A DETERMINATION OF THE DEGREE OF CONSTANCY IN THE NUCLEI OF CERTAIN ORGANS IN HYDATINA SENTA.[1]

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CONTENTS.

INTRODUCTION.

As a result of his extensive studies on the cytology of *Hydatina senta,* Eric Martini (1912: 631) maintained that each individual of this rotifer bears a constant total number of 959 nuclei distributed in fixed numbers through the various organs of the body. In other publications, Martini has mentioned the fact that data concerning variability encountered in his studies of cell-constancy have been too meagre to permit of quantitative study. His published results on cell-constancy in *Hydatina* contain no reference to the numbers of individuals examined in reaching his conclusions of definiteness in cell or nuclear numbers. A. F. Shull (1918), in an attempt to determine the validity of Martini's claim of constancy in histological elements, has made a statistical study of the nuclei in the vitellaria and gastric glands of *Hydatina.* Contrary to the findings of Martini, Shull has recorded a relative "inconstancy" in the numbers of the nuclei in the organs which he investigated. Thus, Shull failed to find eight nuclei an immutable condition for the vitellarium, for in 4 per cent. of his 245 specimens observed an aberrant number was recorded.

Immediately following the appearance of Martini's monograph the present writer became interested in checking over certain minor details of his findings from materials which had been pre-

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pared for class study. A preliminary examination of more than 100 individuals of *Hydatina* failed to reveal any deviation from the usual numbers of nuclei in either the vitellarium or the gastric glands. The same slides were used in a laboratory class in advanced invertebrate zoölogy composed chiefly of university juniors and seniors. For six years each member of this class, averaging about 15 students per year, was required to note and report upon the numbers of nuclei found in the vitellaria of specimens examined. The assignment has always been worded in such a manner as to place a premium upon the discovery of unusual numbers of nuclei, without giving the students any previous hint of the work on cell-constancy. There has never been a verified instance of other than eight nuclei reported by members of these classes. Rather frequently nine nuclei have been reported but in every instance in which a check has been made the ninth nucleus has been demonstrated to belong to the adjacent ovary and readily distinguishable from the nuclei of the vitellarium. The data included in these student observations have not been included in the tabulated results presented later in this paper but comprise a very considerable bulk of evidence of the infrequency of variations in the numbers of nuclei in the material under consideration.

Stimulated by the publication of conflicting observations by Shull, the writer has decided to bring together and to extend his original observations on this phase of the problem of the nuclear numbers in *Hydatina*.

Much of the data available for the statistical study of range in variability are due to the careful assistance of Miss Roselle Karrer who examined 550 individuals of *Hydatina* and by means of a mechanical stage recorded the location of all specimens which displayed peculiarities of any sort. A critical examination of all unusual specimens was made independently by Miss Karrer and the writer, and in no instance was there found any ground for disagreement in the interpretation of the evidence presented by the specimen.

Materials.

The materials upon which these observations are based comprise a series of slides, each containing numerous stained, whole

mounts of *Hydatina senta* prepared by Powers and Powers of Lincoln, Nebraska. The excellence of the cytological details displayed by these mounts speaks well for the methods employed in their preparation. Nuclei of the vitellarium, stomach, gastric

TEXT-FIGURE *A*. Photograph of a whole mount of *Hydatina*. The vitellarium is the large gland, with conspicuous nuclei, near the center of the body. The gastric glands show as bean-shaped organs anterior to the vitellarium and attached to the sides of the stomach.

glands, intestine, and some other structures are readily observable and sharply differentiated from other structures.

The ultimate source of the cultures from which these mounts were made has not been determined. According to Shull's observations (1918: 460) this does not seem to be of any great importance for deviation in nuclear numbers seems to be inherent in the various cultures examined by him. Age, culture media, and

male and female producing strains are the factors specifically considered by Shull in connection with his generalization that there are "no striking differences in the numbers of aberrant glands under different circumstances."

Serial sections were used by Shull in securing his evidence. Numerous sources of probable error are inherent in data resulting from the study of serial sections. Practically all of these are eliminated by the use of well prepared toto mounts. For many features in a work as comprehensive as that of Martini whole mounts would be entirely precluded for the study of finer details of minute structures, but in the study of gross structures such as the vitellaria and gastric glands physical limitations do not preclude the possibility of securing perfect data.

Variability in the Nuclei of the Vitellaria.

In the first 210 individuals examined by the writer each one had 8 nuclei in the vitellarium. At first this was considered as a fair instance of random sampling offering conclusive evidence of absolute constancy in the number of nuclei. Later, in an examination of 560 additional specimens, 3 individuals with aberrant numbers of nuclei in the vitellaria were discovered. Two of these vitellaria possessed 10 nuclei each and the remaining one 12. Thus in 770 specimens examined less than 0.4 per cent. displayed an abnormal number of nuclei in the vitellarium. Contrasting these observations with those of Shull as shown in the following table, two points are especially worthy of note: (1) No individual with less than 8 nuclei in the vitellarium has been observed by the present writer and (2) in no instance has an odd number of nuclei been observed.

Table I.

Frequency of Different Numbers of Nuclei Observed in the Vitellaria of *Hydatina senta.*

Number of nuclei	5	6	7	8	9	10	11	12
Times observed by Shull	1	0	5	235	3	1	0	0
Times observed by Van Cleave	0	0	0	767	0	2	0	1

The plan of the arrangement of the nuclei in the vitellarium of *Hydatina* is not immutable. There is, however, a strong ten-

dency for the nuclei to occur in definite arrangement. Before the gland has reached its full functional stage in development the nuclei (Fig. 2) frequently assume the same definite orderliness of arrangement characteristic of the mature female (Fig. 1). Typically, the 8 nuclei are arranged in the form of a letter V extending across the body with the base of the V at the lateral margin that is contiguous to the ovary. In such instances there is an anterior row of 4 nuclei, a posterior row of 3, and a single nucleus lying near the margin midway between the two rows. This usual arrangement of the nuclei is described because it seems to throw some light upon the question of the interpretation of the aberrant individuals that have been observed in the course of this study.

With but three individuals displaying aberrant numbers of nuclei in the vitellaria the writer realizes that the material is too meagre to permit of broad generalizations. Since, however, all three of these specimens have so many characteristics in common, it seems that the line along which the abnormal condition has been reached must have been fairly uniform. In the two individuals having 10 nuclei in each vitellarium (Figs. 7 and 8), there is strong evidence that the two accessory nuclei have resulted from a division of two nuclei in the region of the ovary. Thus each transverse row contains an additional nucleus. Arrangement of the nuclei in the vitellarium having 12 nuclei (Fig. 9) very strongly supports the hypothesis that this condition has arisen from the typical vitellarium with 8 nuclei through a supplementary division of two nuclei of each transverse row thereby adding 4 nuclei to the normal number. The time at which this division occurred cannot be determined from an examination of the specimens. In any of the three instances mentioned above the non-conformity of nuclear numbers may have been brought about either by the failure of certain nuclei to lose their power of mitotic division at the accustomed stage in the development of the organ or through some sort of stimulation of certain nuclei of a normal mature organ to undergo either mitotic or amitotic division. Evidence observed in the course of this study seems to point toward a late amitotic division occurring in the mature organ.

Since the past history of the specimens under consideration has not been available data concerning the age of individuals and source of the strains are not directly obtainable. Consequently the conditions operating to produce the abnormal glands are not known. In addition to the three instances of abnormal numbers of nuclei in the vitellarium mentioned above, numerous other variations from the usual structure have been observed. Frequently the nuclei are heavily vacuolated (Figs. 3, 4, 5 and 8). An extreme example of vacuolization is shown in Fig. 3 in which one nucleus has attained such a high degree of vacuolization that its chromatic material is highly dispersed and the nucleus greatly swollen.

A more common type of abnormality, possibly associated with advanced age and apparently of significance in considering the problem of increased numbers of nuclei, is that of change in form of the nuclei. Development of irregularity of outline and elongation with accompanying constrictions are two of the more common types of such anomalies. Figs. 4, 5, 6 and 7 show characteristically constricted, lobed, angular, indented, and twisted nuclei. These varying forms all give the appearance of adaptations described in the literature on cytology for increase in nuclear surface necessitated by some change in the normal activity of the gland. In this specific instance no experiments have been performed to determine the conditions responsible for this attempt at readjustment of nuclear surface to meet increased demands. It does seem significant that the changes in form of the nuclei closely resemble conditions observed in other tissues the nuclei of which are undergoing amitotic fragmentation. From nuclei such as shown in Figs. 4 and 6 it is no very great step to a separation of the constricted parts into independent nuclear masses.

Modifications of an identical type have been observed in different genera of Acanthocephala. As shown by the writer (Van Cleave 1914) members of the family Neoechinorhynchidæ display six giant nuclei in the entire subcuticula. In all genera of this family these subcuticular nuclei are definitely limited ovoid or elliptical masses. In members of the genus *Quadrigyrus* (Van Cleave 1920) these same subcuticular nuclei are of two types,

those in the anterior region of the body being identical in form with those just described for the Neoechinorhynchidæ while those in the posterior region are coarsely dendritic in nature, composed of an elongated central mass from which a considerable number of projections are given off along the channels of the lacunar system. Nuclei of this second type are distinctly intermediate between the form characteristic of the Neoechinorhynchidæ and those described for the species *Echinorhynchus thecatus*. In this last named species the subcuticular nuclei are finely dendritic, extending as fine branches over a considerable area. In some species of the genus *Echinorhynchus* and of the genus *Pomphorhynchus* this dendritic condition has gone to such an extreme that the small nuclear masses are joined by only fine threads and in many places these communicating threads have not been detected. In this last named condition the nuclear material has the appearance of being distributed in small isolated nuclear masses throughout certain regions of the subcuticula. Thus in this group of parasitic worms the subcuticular nuclei present a finely graded series of changes from the simple rounded or ovoid nuclei retained directly from the embryonic condition to a highly fragmented condition in which there is little or no regularity in the numbers and arrangement of the particles resulting from the fragmentation. Consequently in Neoechinorhynchidæ there is a constancy in the number of the subcuticular nuclei throughout the life of the individual while in the most of the other Acanthocephala late embryonic or post-embryonic fragmentation of the nuclei disrupts the constancy in numbers of histological elements.

That a similar amitotic division might be the explanation of increased numbers of nuclei in the aberrant vitellaria of *Hydatina senta* seems highly probable. Moreover, the abnormal individuals observed in this study seem to indicate that definitely localized stimuli operate in inducing further divisions of the nuclei, for not all of the nuclei of the vitellarium are affected. In all three of the aberrant specimens observed the increase in the number of the nuclei has apparently been in the region of the vitellarium immediately in contact with the ovary. The exact nature of the stimulating agent has not been determined but it seems

probable that some change in the vitellarium may be induced by or correlated with changes in the adjacent ovary.

The glands which have 10 nuclei each seem to offer distinct evidence that the additional nuclei have been derived by the division of one nucleus from the anterior and one from the posterior row of a normal individual (Figs. 7 and 8). If two nuclei of each row in a normal individual were to undergo division 12 nuclei would result as is the case in one individual observed (Fig. 9).

Nuclei of the Gastric Glands.

Shull found 9 out of 120 gastric glands which he examined carrying numbers of nuclei other than 6, the characteristic number. According to his records 7.5 per cent. of the glands fail to support the idea of constancy in the numbers of nuclei. By using the same whole mounts described earlier in this paper the writer has secured data on the nuclei of 435 gastric glands, not a single one of which displayed any deviation from the 6 nuclei characteristic for that organ. A comparison of the two sets of data is clearly presented in the following table.

Table II.

Frequency of Different Numbers of Nuclei Observed in the Gastric Glands of *Hydatina senta*.

Number of nuclei	4	5	6	7
Times observed by Shull	1	7	111	1
Times observed by Van Cleave	0	0	435	0

According to Shull's statement (1918: 463) all of the various conditions of culture medium, age, and male or female producing strains seem to produce aberrant numbers of gastric gland nuclei. In the case of male producers he examined only 36 individuals and found none with aberrant numbers of nuclei, but also mentioned the fact that in view of the small numbers examined this circumstance may be insignificant.

Conclusions.

Referring to the solution of the problem of cell-constancy, Shull (1918: 464) has said: "there is no need of complicating

this solution by assuming a degree of constancy that does not exist." In speaking of the results of his own work on cell-constancy in *Hydatina* he has maintained that he has obtained "A measure of its exactitude" as expressed in his finding 4 per cent. of individuals bearing inconstant numbers of nuclei in the vitellarium and 7.5 per cent. showing aberrant numbers of nuclei in the gastric glands.

In contrast with these observations stand those included in the present paper wherein a much greater number of individuals were examined, and yielded less than 0.4 per cent. inconstant vitellaria and absolute constancy in the gastric glands.

The complete absence of subnormal numbers of nuclei from the data presented by the present writer seems especially significant in that it indicates that the mechanism operating to bring about constancy of nuclear numbers has operated to its full extent and that any abnormalities represent an inconstancy secondarily imposed. Reductions in nuclear numbers might also result from secondary causes but the writer has seen no evidence of factors operating in the regions studied to bring about a reduction in the numbers of nuclei.

As a result of his observations, Shull seems to have full conviction that the tendency to vary is inherent in the nuclear numbers of *Hydatina* regardless of circumstances such as culture medium and strain. If his conclusions in this regard are valid, either some factor or group of factors not considered by Shull have been operating to suppress the inherent tendency to vary in the particular culture studied by the writer or physical difficulties involved in reconstructing and counting the nuclei from serial sections have introduced an element of error into his results.

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Plate I.

All drawings were made from whole mounts with the aid of a camera lucida and at a uniform magnification.

The vitellarium is viewed from the ventral surface in all instances except in Figs. 4 and 7, which are drawn from the dorsal surface.

Fig. 1. Vitellarium of normal mature female, showing usual form of the gland and characteristic number and arrangement of the nuclei.

Fig. 2. Very small vitellarium from a presumably young female.

Fig. 3. Vitellarium showing characteristically vacuolated nuclei, one of which has the chromatic substance so much dispersed that it is distinctly paler in color.

Fig. 4. Vitellarium showing lobed, angular and constricted nuclei.

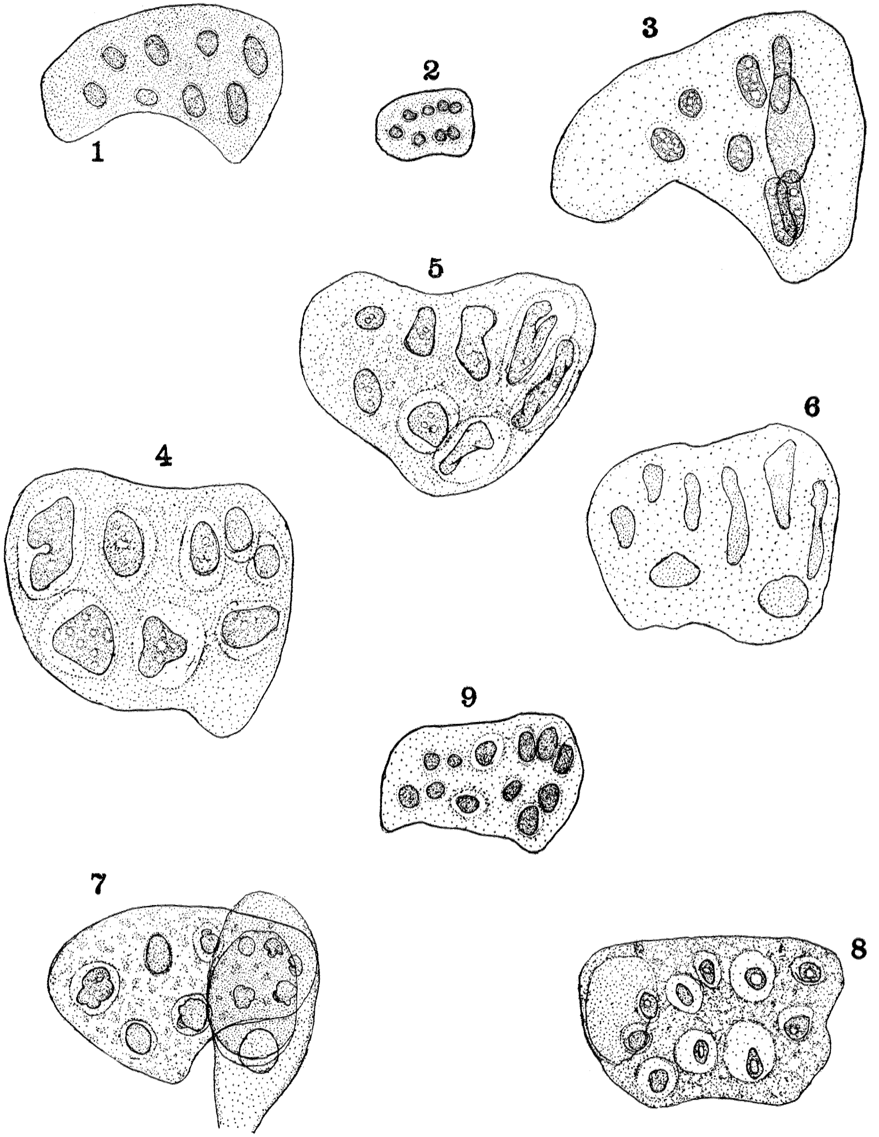
Fig. 5. Vitellarium with peculiarly twisted and cleft nuclei.

Fig. 6. Abnormal vitellarium with greatly elongated and constricted nuclei.

Fig. 7. Abnormal vitellarium with 10 nuclei. In this drawing the ovary is shown overlying the vitellarium on the right side.

Fig. 8. Abnormal vitellarium with 10 nuclei. Part of the ovary is shown underlying the vitellarium on the left of this drawing.

Fig. 9. Abnormal vitellarium with 12 nuclei.



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